

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Michael Knoblauch, et al.  
Serial No: 10/605,104  
Filing Date: 9/9/2003  
Title: Forisomes, Method for Their Isolation, and Their Use as a  
Molecular Working Machine  
Examiner: Marsha M. Tsay  
Art Unit: 1656

**Commissioner for Patents**  
**Alexandria, VA 22313-1450**

**DECLARATION PURSUANT TO 37 CFR 1.132**

I, Prof. Dr. Dirk Prüfer, Machabaerstr. 57, Cologne, Germany, am one of the co-inventors of the instant application. I received my PhD degree ("Dr.") in 1992 at the University of Cologne and was Head of the Department "Functional and Applied Genomics" and Head of the Section "Molecular Biology" at the assignee, Fraunhofer-Gesellschaft, at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Aachen and Schmollenberg, Germany, between 1999 and 2004. As from December 2004, I am Full Professor for Molecular Biotechnology of Plants at the Institute for Biochemistry and Biotechnology of Plants at Münster University, as well as Head of Department "Functional and Applied Genomics" at the above Fraunhofer Institute.

The main field of my scientific research is directed to forisomes since 2000.

The amino acid sequence SEQ ID NO:2 as described in our above mentioned application is a sequence which occurs highly conserved in all P1 proteins. These proteins are responsible for the mechanical behavior ("stopcock") of the forisomes in fabaceae. In order to demonstrate that SEQ ID NO:2 occurs in and therefore is an inherent feature of all P1 proteins, i.e. that it can be assigned to the P1 protein only, the amino acid sequence of five P1 proteins, derived from four different fabaceae plants, is listed on the attached sheet. As examples, EGFDIAFK = Glu-Gly-Phe-Asp-Ile-Ala-Phe-Lys is found in line 11 of each of the sequences; these five sequences are publicly accessible via the US NCBI gene database:

MtP1.1 = 1. Variant of Medicago trunculata = MtSEO-F1

<http://www.ncbi.nlm.nih.gov/protein/ABV32455.1>

MtP1.2 = 2. Variant of *Medicago trunculata* = MtSEO-F4

<http://www.ncbi.nlm.nih.gov/protein/ADN32805.1>

GmP1 = *Glycine max.* = GmSEO-F4

<http://www.ncbi.nlm.nih.gov/protein/ADN32792.1>

VfP1 = *Vicia faba* = Vffor1

<http://www.ncbi.nlm.nih.gov/protein/ABV32453.1>

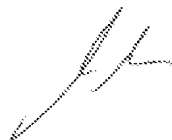
CgP1 = *Canavalia gladiata* = Cgfor1

<http://www.ncbi.nlm.nih.gov/protein/ABV32453.1>

About the forisomes of *Medicago trunculata*, I have reported with co-authors in G.A.Noll et al., *Plant Mol Biol* (2007), 65:285-294 (copy attached). The provisional name of the protein as used in our patent application ("P1") had been corrected therein to be in line with systematic designations used in this field; however, since the term "for" was already occupied, it was later amended into "SEO" ("Sieve Element Occlusion"), see the paper e.g. B. Müller et al. in *Appl. Microbiol. Biotechnol.* (2010) 88:689-698 (copy attached), where we report on the possibility to obtain recombinant artificial forisomes from expression of MtSEO1 and MtSEO4 in plants and yeast.

I herewith declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and that I am warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Signed this \_\_\_\_4\_\_\_\_ day of October 2010



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(signature)

DIRK PRÜFER  
(printed name)

MfP1.1 MS-LGN-----G--KLLNPFPLDESQYLKRV  
 MfP1.2 MS-LNHLGSA-----TATUSHLAQKNATGSLQ--KA-NFLPNPFPLHGPQYLKRV  
 CqP1 MS-DEK-----PQCK--ALLPNPFYLSGSHLEKV  
 VEP1 MS-FSNSRAARTGTLVQMGSGSTNHLIQKATATSGMPPHKANWYLAHGFELHDSHILLEV  
 GmP1 MAQLNGST-G-----STTLYSRSGTTSTPR-RA--SLPNPFULTSDQYLDIV  
 SEQ NO:2 -----  
  
 MfP1.1 YLNLKNDGKNGKGVLPKHLGNVILKTRL-----AESRAEPEPEFTT  
 MfP1.2 YLTHVTLDKPCDTHIYFELVAGVVLQTTI-----KLSVTSFKPEPEFT  
 CqP1 YLTHYTDKACDRSTFLNLMSTVIMHSTKIVETFTLKPDVNHGTFGSKMLITSEFKPSFHK  
 VEP1 YLNMVDEGFOSTDEIPELVSTHLLQGNL-----QYFTGSHKEDFT  
 GmP1 YLNLNDEDTCDTDMLYNLVGNIVLAKGG-----FISAAGFYFDFT  
 SEQ NO:2 -----  
  
 MfP1.1 LKLTSCQMITTFEGEKYVHQTTWILLQQLKTYSDAKALIALAAPTLSYHLLYLTETST  
 MfP1.2 LKLYSCQMITTTHNDHCVKQTTLLQLKLSYSDAKALITLAAFTLEYGNVYQLNRYT-  
 CqP1 LKLLSCQMITTFEGLENARQTTMBITLQQLSFSFWERKALIALAAPLEYGNFRHLYQLP-  
 VEP1 LKLLSCQMITTTRSVAMCVHQTTLWLLQNLKRYSDAKALITLAAFTLEYGNVYQLNRYT-  
 GmP1 LKLTSCQMITTATAGAAKCVHQTTWILLQNLKRYSDAKALIALAALSLEYGSPVHLTQPP-  
 SEQ NO:2 -----  
  
 MfP1.1 GSDQLVHSLKILSLQIQKIKVTV--STDLVELIMDVLLMINENATRSQGYHTLEWVGLGD  
 MfP1.2 TTTDLGNLKLVLINQVQTRKISH--DVTLLVRYIVDMLRLNWTWSDAGVDVDFVPALED  
 CqP1 PSDQLGNLKLINQVQTRKIPITIDINNSVVLNMEVVGKINNNQVWLAGGYDTSTVPALED  
 VEP1 ATDTEGSHLQQLGQVQTRKIST--GEFELVNP1VKKLIMLKEKAAWSABQYDFEDVPALE  
 GmP1 TNDVLGNLKLINQVQTRKIAS--R-VQELVMYVQVQVQHNENATYARQGYDFEDVPALE  
 SEQ NO:2 -----  
  
 MfP1.1 ALQEIIVAVVWYIASTVAATONLIGVGDYTLSDPYEKLVFVGLKLAENHAKLSKQITDVE  
 MfP1.2 ALQELFVAVVWYIASTVASTGHLVGVSDYKLSAYKERLSKVVESELVNHATCEKQIPNVD  
 CqP1 ALQELFVAVVWYIASTVASTGHLVGVSDYKLSAYKERLSKVVESELVNHATCEKQIPNVD  
 VEP1 ALQEIIVAVVWYIASTVASTONLVGVSDYKLSAYKERLSKVVESELVNHATCEKQIPNVD  
 GmP1 ALQELFVAVVWYIASTVASTONLIGVGNXKLSVETTELSTAVHKLTHRLTYVKEQTANVA  
 SEQ NO:2 -----  
  
 MfP1.1 EYLKRNKALSHFKYITDFLKLLIQ---BNG--DNLLIYDGTTHNETDTEVPKDKYVLLPISG  
 MfP1.2 ELTSRTNRYKPKPDIYDGLKALIH---RNGSTDTFQIYQONVQVMSGLDTFKQKHVLLFTSG  
 CqP1 EYMRKRNFKPKPDIYDGLKALIH---RNGSTDAQVFGSGQTKVSEVEFKQKHVLLFTSG  
 VEP1 EYMRKRNFKPKPDIYDGLKALIH---RNGSTDAQVFGSGQTKVSEVEFKQKHVLLFTSG  
 GmP1 EYLTIANITDTPKDIYDGLKALLYPQONGARNPKIPDGTNLVKGIEVEFKQKHVLLFTSG  
 SEQ NO:2 -----  
  
 MfP1.1 LNFVDEEILLNBIHHLQGMFQVI--EGYKNEDEFTLWTFINDV---DEQKINPEGLNKEI  
 MfP1.2 LNFVDEEILLNBIHHLQGMFQVI--EGYKNEDEFTLWTFINDV---DEQKINPEGLNKEI  
 CqP1 LNFVDEEILLNBIHHLQGMFQVI--EGYKNEDEFTLWTFINDV---DEQKINPEGLNKEI  
 VEP1 LNFVDEEILLNBIHHLQGMFQVI--EGYKNEDEFTLWTFINDV---DEQKINPEGLNKEI  
 GmP1 LNFVDEEILLNBIHHLQGMFQVI--EGYKNEDEFTLWTFINDV---DEQKINPEGLNKEI  
 SEQ NO:2 -----  
  
 MfP1.1 RFTAVEYFSELFGILRLR---ENLNTSKPIFVVLGSLGKNNNDAMDLYFQKSIDALFPR  
 MfP1.2 RFTAVEYFSELFGILRLR---ENLNTSKPIFVVLGSLGKNNNDAMDLYFQKSIDALFPR  
 CqP1 RFTAVEYFSELFGILRLR---ENLNTSKPIFVVLGSLGKNNNDAMDLYFQKSIDALFPR  
 VEP1 RFTAVEYFSELFGILRLR---ENLNTSKPIFVVLGSLGKNNNDAMDLYFQKSIDALFPR  
 GmP1 RFTAVEYFSELFGILRLR---ENLNTSKPIFVVLGSLGKNNNDAMDLYFQKSIDALFPR  
 SEQ NO:2 -----  
  
 MfP1.1 KQDGYELTQKNNKFWLWTERVNLGIYVKGDAYIETYGSDKNNWQDFTLALBETKRNHETI  
 MfP1.2 KQDGYELTQKNNKFWLWTERVNLGIYVKGDAYIETYGSDKNNWQDFTLALBETKRNHETI  
 CqP1 KQDGYELTQKNNKFWLWTERVNLGIYVKGDAYIETYGSDKNNWQDFTLALBETKRNHETI  
 VEP1 KQDGYELTQKNNKFWLWTERVNLGIYVKGDAYIETYGSDKNNWQDFTLALBETKRNHETI  
 GmP1 KQDGYELTQKNNKFWLWTERVNLGIYVKGDAYIETYGSDKNNWQDFTLALBETKRNHETI  
 SEQ NO:2 -----  
  
 MfP1.1 LRADWIEHYHLGKDE---PRIVERWIEIESEKL--KHKQDSEILCEYQHVKNLLCLNQD  
 MfP1.2 LRADWIEHYHLGKDE---PRIVERWIEIESEKL--KHKQDSEILCEYQHVKNLLCLNQD  
 CqP1 LRADWIEHYHLGKDE---PRIVERWIEIESEKL--KHKQDSEILCEYQHVKNLLCLNQD  
 VEP1 LRADWIEHYHLGKDE---PRIVERWIEIESEKL--KHKQDSEILCEYQHVKNLLCLNQD  
 GmP1 LRADWIEHYHLGKDE---PRIVERWIEIESEKL--KHKQDSEILCEYQHVKNLLCLNQD  
 SEQ NO:2 -----  
  
 MfP1.1 PQQWVILLKSGYVYLLGSGPMYQTLADPDINKDAVLQSGFDIAFKYDYDKVKDTYV-  
 MfP1.2 PQQWVILLKSGYVYLLGSGPMYQTLADPDINKDAVLQSGFDIAFKYDYDKVKDTYV-  
 CqP1 PQQWVILLKSGYVYLLGSGPMYQTLADPDINKDAVLQSGFDIAFKYDYDKVKDTYV-  
 VEP1 PQQWVILLKSGYVYLLGSGPMYQTLADPDINKDAVLQSGFDIAFKYDYDKVKDTYV-  
 GmP1 PQQWVILLKSGYVYLLGSGPMYQTLADPDINKDAVLQSGFDIAFKYDYDKVKDTYV-  
 SEQ NO:2 -----  
  
 MfP1.1 KQPCETINVDANTGNVLAITSCPNPTCGRVMEVSGVHYKCHRDADAAPQNGHT  
 MfP1.2 KQPCETINVDANTGNVLAITSCPNPTCGRVMEVSGVHYKCHRDADAAPQNGHT  
 CqP1 KQPCETINVDANTGNVLAITSCPNPTCGRVMEVSGVHYKCHRDADAAPQNGHT  
 VEP1 KQPCETINVDANTGNVLAITSCPNPTCGRVMEVSGVHYKCHRDADAAPQNGHT  
 GmP1 KQPCETINVDANTGNVLAITSCPNPTCGRVMEVSGVHYKCHRDADAAPQNGHT  
 SEQ NO:2 -----